G-protein diseases furnish a model for the turn-on switch

Taroh Iiri*†, Zvi Farfel‡ & Henry R. Bourne*

** Departments of Cellular and Molecular Pharmacology and Medicine, University of California, San Francisco, California 94143-0450, USA*
‡ Laboratory of Biochemical Pharmacology and Department of Medicine E, Sheba Medical Center, Tel Aviv University, Tel Hashomer, Israel

How does a trimeric G protein on the inside of a cell membrane respond to activation by a transmembrane receptor? G-protein mutations in patients with hypertension and inherited endocrine disorders enhance or block signals from stimulated receptors. In combination with three-dimensional crystal structures and results from biochemical experiments, the phenotypes produced by these mutations suggest a model for the molecular activation mechanism that relays hormonal and sensory signals transmitted by many transmembrane receptors.

Trimeric ($\alpha\beta\gamma$) G proteins relay signals from transmembrane receptors to intracellular enzymes and ion channels, thereby mediating vision, smell, taste and the actions of many hormones and neurotransmitters[1,2]. Much effort has been devoted to elucidating the receptor-triggered 'turn-on' step of the GTPase cycle (Fig. 1): what happens at the atomic level when a receptor turns a G protein on, promoting exchange of GTP for GDP bound to the Gα subunit, followed by dissociation of Gα·GTP from Gβγ and of both subunits from the receptor?

Figure 2 depicts a G-protein trimer in its probable orientation[3,4] relative to a G-protein-coupled receptor and the plasma membrane. The receptor switch is thought to be composed of seven α-helices folded into a bundle that spans the membrane[4] (reviewed in refs 5–8). We shall focus here on the crucial problem posed by the orientation of the Gαβγ trimer relative to the receptor (Fig. 2): cytoplasmic loops of most receptors (not depicted in the figure) are too short to reach more than halfway to the site where GDP is bound, about 30 Å from the plasma membrane[1,8]; how then does the receptor act at a distance to cause release of bound GDP? We propose a speculative but testable model, in which the activating message is relayed from the receptor to the GDP-binding pocket of Gα by two complementary routes. Receptor-catalysed GDP/GTP exchange uses a switching mechanism and unique structural features that differ greatly from those that regulate GDP/GTP exchange in monomeric GTPases, such as Ras and the elongation factor (EF) Tu of protein synthesis. Just as G-protein mutations in human disease opened the way[2,9–12] to understanding the G-protein 'turn-off' mechanism at the atomic level[2,11–16], other genetic diseases–ranging from rare endocrine disorders to hypertension—furnish critical clues to understanding the turn-on mechanism.

Action at a distance

Crystals have revealed three-dimensional (3D) structures of the substrate and product of the GDP/GTP exchange reaction (Fig. 1) catalysed by receptors. These are, respectively, the GDP-bound $\alpha\beta\gamma$ complex and the GTP-bound Gα and uncomplexed Gβγ subunits[3,15,17–22]. To understand the catalytic mechanism, however, we need a model of a G protein with an empty guanine-nucleotide-binding site ($\alpha_e\beta\gamma$ in Fig. 1). Such a model remains elusive because the key intermediate conformation is thermally labile in the absence of the catalyst, as might be expected. The catalyst—an activated receptor—does stabilize $\alpha_e\beta\gamma$, but receptors have proved hard to crystallize.

The 3D structure of α·GDP·βγ (Fig. 2), the starting point of the GDP/GTP exchange reaction, reveals the 'action at a distance' problem faced by the receptor. The guanine nucleotide (yellow) is cradled between two domains of the Gα subunit: one domain (grey) resembles those of Ras and other monomeric GTP-binding proteins; the other (orange) is an α-helical domain not found in other GTPases[2]. Nucleotide-binding loops of the former domain, connecting β-strands and α-helices, share conserved amino-acid sequences with those of Ras and EF-Tu; loops at the opposite ends of the same β-strands and α-helices contact the receptor[23–25]. Gβ binds to the Ras-like domain of Gα.

The complex (not shown) of two bacterial elongation (EF) factors, Tu and Ts, has provided the only available 3D structures[26,27] of the 'empty nucleotide pocket' stage in a GDP/GTP exchange reaction. Figure 3 highlights, in blue and black, residues of Gα·GDP that correspond to those of EF-Tu that contact the exchange catalyst EF-Ts. The virtual footprint of Ts on Gα does not overlap with the Gα surface (red) available to the receptor[23–25] (red). Instead, EF-Ts catalyses exchange by mounting a comprehensive attack on the nucleotide-binding pocket itself, poking a phenylalanine residue directly into it, releasing bound Mg^{2+}, and disrupting interactions of two loops with the β-phosphate and the guanine ring of GDP[26,27]. Finally, Ts creates an exit route for the nucleotide by nudging yet a third loop out the way; this loop, cognate to the β3/α2 loop of Gα, contains the black residues in Fig. 3.

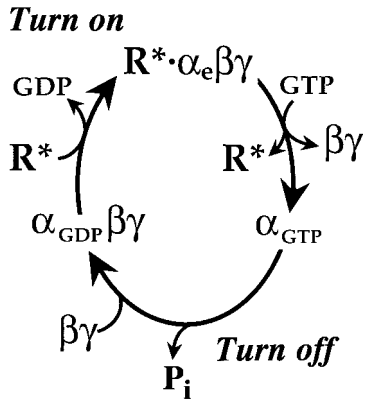

Figure 1 The GTPase cycle of trimeric G proteins. The 'turn-on' step begins when the activated receptor (R*) associates with the trimer of (α·GDP·βγ), causing dissociation of GDP. Then GTP binds to the complex of R* with the trimer in its 'empty' state (α_e·βγ), and the resulting GTP-induced conformational change causes α·GTP to dissociate from R* and from βγ. After the 'turn-off' step (hydrolysis of bound GTP to GDP and inorganic phosphate, P_i), α·GDP reassociates with βγ.

† Present address: Fourth Department of Internal Medicine, University of Tokyo School of Medicine, 3-28-6 Mejirodai, Bunkyo-tu, Tokyo 112, Japan.

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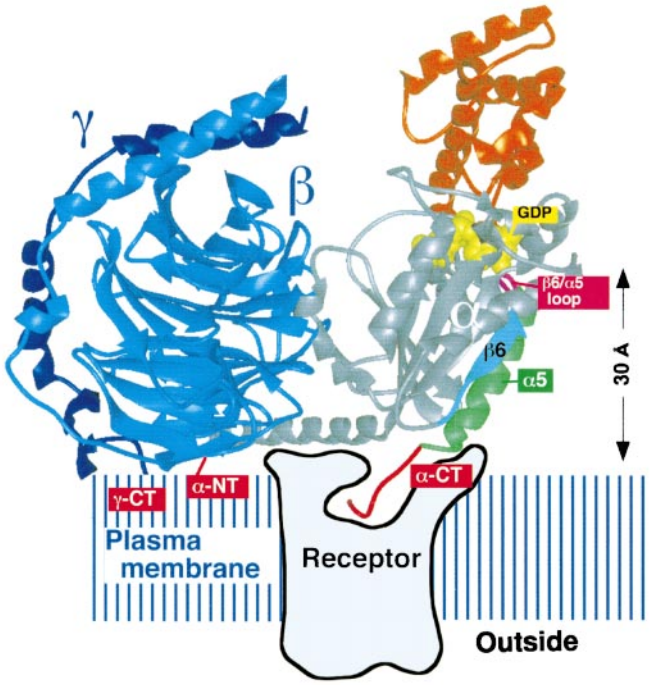

Figure 2 The postulated[3,8] orientation of a G-protein trimer to a transmembrane receptor. The carboxy-terminal (CT) tail interacts with the receptor. Lipid modifications of the amino termini (NT) of Gα and Gγ attach the trimer to the plasma membrane[3]. The β6 strand and the α5 helix are postulated to transmit receptor-induced conformational change to the guanine ring of GDP, which contacts the β6/α5 loop. The trimer structure is based upon that of G_t (ref. 3).

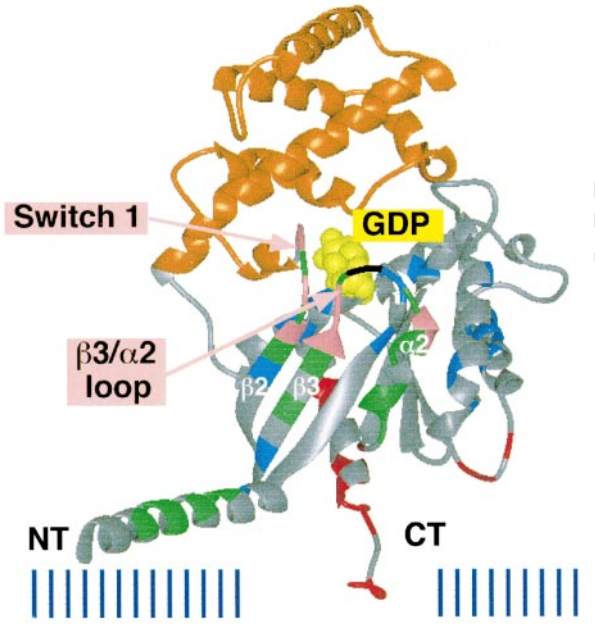

Figure 3 Surfaces of Gα·GDP that may play a key role in the GDP/GTP exchange reaction. The Gα·GDP component of the G_t trimer[3] is rotated anticlockwise 90° about a vertical axis with respect to the orientation shown in Fig. 2; its Gβγ-binding surface faces the viewer. Coloured segments indicate amino acids that contact Gβγ (green)[3], are thought to contact the receptor (red)[24], are cognate to the residues of EF-Tu contacted by its exchange catalyst EF-Ts (blue)[27], or serve as contact points for both Gβγ and EF-Ts (black). Pink regions demarcate the lip of a potential exit route for GDP, comprising the switch-1 loop (which connects the α-helical and Ras-like domains of Gα), the β3/α2 loop, and part of the α2 helix.

We propose (see below) that the receptor uses Gβγ to open a cognate exit route in Gα during receptor-promoted GDP/GTP exchange. The footprint of Gβ on Gα (green and black in Fig. 3)[3,21] partially overlaps the virtual footprint of EF-Ts; two cognate residues (black in Fig. 3) are touched by both proteins. A major part of the Gβγ-contacting surface of Gα is composed of four consecutive secondary structures, including the 'switch 1' loop connecting the α-helical domain to the β2 strand, the β2 and β3 strands, and the α2 helix. The 'lip' (pink in Fig. 3) of this contact surface, switch 1 and the β3/α2 loop, occludes the exit route used by EF-Ts to release GDP from EF-Tu.

Regulation of GTP release by Gα- and Gβγ

The biochemical phenotype of one human Gα mutant strikingly imitates the receptor-catalysed release of GDP. The patients, suffering from a combination of two rare endocrine diseases, carry a point mutation in codon 366 of the gene encoding the α-subunit of the stimulatory regulator of adenylyl cyclase, G_s (ref. 28). The alanine normally found at this position, located in the β6/α5 loop (magenta in Fig. 2), makes a van der Waals contact with the guanine ring of the guanine nucleotide[22]. The slightly larger side chain of the serine substituted at this position (residue 366) causes the mutant α_s-A366S protein to release GDP spontaneously, at a rate 80 times faster than the wild-type subunit (α_s-WT). In the testis, rapid release of GDP mimics stimulation by gonadotropin receptors, accelerating GTP binding and G_s activation; the result, in males, is precocious puberty caused by autonomous production of testosterone (testotoxicosis). In other tissues, however, the patients show the diminished G_s-dependent hormone responses that are characteristic of type I pseudohypoparathyroidism. The defective hormone responses result from the thermolability of α_s-A366S at body temperature (37 °C) and its stability at testis temperature (about 34 °C). The conserved alanine mutated in these patients is a potential control point for regulation of GDP release. Its replacement by other amino acids accelerates GDP release from other Gα subunits ($G\alpha_{i2}$ and $G\alpha_z$; P. Wilson, J. Morales, T.I. and H.R.B., unpublished results) and also from Ras[29].

How could the receptor act at a distance to deform the β6/α5 loop of Gα? Considerable evidence (from mutations, peptides and covalent modification by a bacterial toxin[23,25,30,31]) points to the carboxy-terminal tail (red in Fig. 2) of Gα as an important site for interaction with the receptor, achieved perhaps[8] by insertion into a cavity[32] formed by the seven-helix bundle (Fig. 2). The tail is located at the end of the α5 helix (green in Fig. 2)—that is, at the other end of the helix from the β6/α5 loop. Receptors interact also with side chains of residues at the 'membrane end' of β6 (cyan in Fig. 2)[24,25,33,34]; like α5, β6 connects the receptor-binding surface to the β6/α5 loop. Moreover, release of GDP is accelerated by a truncation that removes part of the α5 helix and the C-terminal tail from $G\alpha_o$ (ref. 35). Thus β6 and α5 probably bear at least partial responsibility for communicating the receptor signal to the nucleotide-binding pocket.

By analogy with EF-Ts acting on Tu, we and others[12] propose that Gβγ plays a second, complementary role in communicating the activating signal from receptor to the nucleotide-binding site. The scenario is simple: the membrane-facing side of the Gαβγ complex contains a prominent cavity, previously noted[3,8], between Gα and Gβγ. The cavity provides an opportunity for loops of the activated receptor to tilt Gβγ and Gα away from one another, causing Gβ–Gα contacts to pull the flexible lip away from a potential exit from

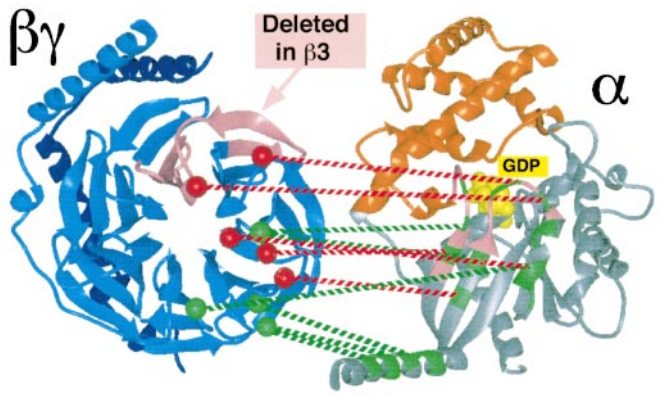

Figure 4 Contacts between Gβγ (left) and Gα·GDP (right). With respect to their orientations in Fig. 2, Gα·GDP and Gβγ are rotated about a vertical axis, anti-clockwise by 90° and clockwise by 60°, respectively. The C-terminal tail of Gα·GDP and the contacts with the receptor and Ts are not shown; pink in Gα indicates a 'lip' that occludes the postulated exit route for GDP (see text). In Gβ, pink indicates the stretch of amino acids deleted in Gβ3-s (ref. 37). The green and red balls indicate Gβ positions where alanine substitutions reduce responsiveness of G_t to activation; of these, five (red balls) do not appreciably reduce the affinity of Gα for binding Gβγ (see text)[36]. Dashed lines connect residues at these mutated positions in Gβ to the α-carbon(s) of the Gα residue(s) with which each makes contact; red dashed lines indicate contacts that appear to be required for receptor activation but not for Gα–Gβγ association; green dashed lines indicate contacts that are important for both functions[36].

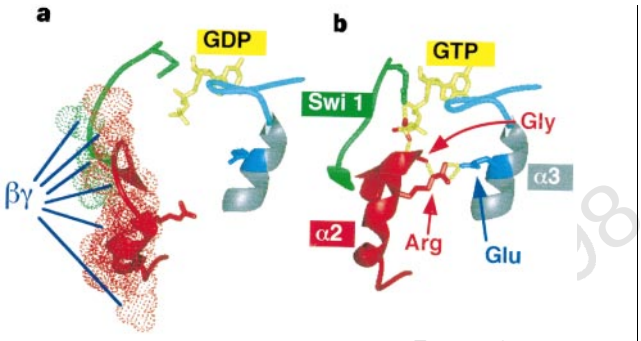

Figure 5 Different conformations of Gα·GDP in the trimer[3] and GTP-bound Gα (ref. 18). **a**, In the trimer, association of Gα·GDP with Gβγ induces movements of the switch 1 loop (Swi1 in **b**; green) and the α2 helix (red) away from the guanine nucleotide and places an arginine (red; see **b**) in the α2 helix out of reach of a glutamate (blue; see **b**) in the α3 helix; residues that contact Gβ in the trimer are sown in a van der Waals representation (red and green dots). **b**, The GTP-bound Gα conformation is stabilized by the intramolecular hasp (yellow dashes) formed by a salt bridge between the arginine residue (red) in the α2 helix and the glutamate (blue) in the α3 helix; the arginine side chain is also linked to the main-chain carbonyl of a conserved glycine in the β3/α2 loop. The guanine nucleotide is shown in yellow, except that oxygens of the γ-phosphate of GTP are in red.

the GDP-binding site. Thus the receptor uses Gβγ as a lever to effect GDP release at a distance.

Considerable evidence supports this scenario. Because receptors interact directly with Gβγ and require Gβγ for efficient activation of Gα (ref. 8), it is not surprising that alanine substitutions at the Gα–Gβ interface[24,36] impair receptor-induced GDP/GTP exchange. More instructive, five alanine substitutions in Gβ inhibit receptor-promoted exchange but do not substantially interfere with binding of Gβγ to Gα (ref. 36). This subset of Gβ–Gα contacts (red dashed lines in Fig. 4) probably plays important roles in receptor-induced GDP/GTP exchange, as suggested by their locations at or near the lip (pink in Figs 3 and 4) of the proposed exit route for GDP. In the unstimulated trimer, these contacts presumably stiffen the lip, enhancing affinity for GDP; a receptor-induced tilt of Gβγ away from Gα, however, could use the same contacts to pull the flexible lip away from the exit.

A recently reported human Gβ mutation[37] produces a 'gain-of-function' G-protein signalling abnormality, perhaps by enhancing the proposed active role of Gβγ in receptor-catalysed activation of G proteins. Aberrant splicing of the transcript of a mutant gene that encodes the β3 member of the Gβ family[37], frequently seen in patients with essential hypertension, leads to production of a short protein, termed Gβ3-s, that lacks an internal stretch of 41 amino acids. Astonishingly, Gβ3-s functions in the signalling machinery of platelets and cultured cells from hypertensive patients and in insect cells expressing the mutant polypeptide[37]. Expression of Gβ3-s is accompanied by enhanced sensitivity of G_i proteins to receptor activation.

The modular structure of Gβ (Fig. 4) probably explains the surprising ability of Gβ3-s to fold and to transmit signals[38]. Gβ isozymes are composed of seven very similar β-sheets surrounding a central hole, like blades of a propeller[19,21]. The aberrant splice excises the equivalent of one propeller blade (pink in Fig. 4), presumably allowing the six remaining blades to adopt a similar fold around the central hole. Despite its diminished girth, the new fold could preserve contacts with Gγ, which stabilizes the Gβ fold[39] and orients the Gβγ dimer with respect to the plasma membrane[3].

How might the Gβ3-s mutation enhance the transmission of conformational change from the receptor to the nucleotide-binding site? Excision of the propeller blade from Gβ would change the positions of critical Gα-contacting residues relative to one another and to the plane of the membrane. The Gα contacts most affected would be in the lip that guards the proposed exit route for GDP (Fig. 4). This hypothesis can be tested biochemically.

A Gα mutation disrupts GTP binding

Release of bound GDP is not enough to activate a G protein; in the second step of the GDP/GTP exchange reaction, GTP must enter the nucleotide-binding pocket of $\alpha_e\beta\gamma$ and trigger the dissociation of Gα, Gβγ and the receptor (Fig. 1). This critical second step in the exchange reaction cannot be automatic, because the receptor must destabilize the guanine-nucleotide-binding site in order to promote release of bound GDP. Each of the complementary mechanisms we have proposed should destabilize binding of GTP as well as GDP; indeed, receptors can increase the rates of dissociation of GTP analogues from G proteins[40]. Normally, however, GTP efficiently replaces GDP in the binding site because the γ-phosphate rescues Gα from receptor-induced instability. The additional binding energy furnished by the γ-phosphate promotes a conformational change that causes Gα to dissociate from βγ and from the receptor; separation from the receptor definitively removes the destabilizing effect.

The essential moving part of this conformational switch is the α2 helix of Gα. In the trimer (Figs 2–4), many residues of this helix and the preceding β3/α2 loop interact with Gβγ (refs 3, 21). In the transition from the trimer (Fig. 5a) to the Gα·GTP conformation (Fig. 5b), the amino terminus of this helix moves about 3 Å closer to the guanine nucleotide; the helix also twists on its axis, exposing a different set of amino-acid side chains. Neither change could occur without dissociation of Gα from Gβγ, which stabilizes both the position and the axial rotation of the α2 helix in the trimer. Thus the transition from $R^*{\cdot}\alpha_e\beta\gamma$ to $R^* + \alpha{\cdot}GTP + \beta\gamma$ (Fig. 1) reflects the outcome of a molecular tug-of-war, staged between Gβγ and the γ-phosphate of GTP, for controlling the position of the α2 helix. The

exact position of the α2 helix in Gα·GTP (Fig. 5b) is specified by a link between an oxygen of the γ-phosphate and the main-chain amide of a conserved glycine in the β3/α2 loop, which precedes the helix[15,17,18].

The link does not suffice for GTP to win the tug-of-war against Gβγ, as shown by an instructive $G\alpha_s$ mutation[41,42], found in a family with type I pseudohypoparathyroidism. This mutation, substituting histidine for a conserved arginine at position 231 (ref. 41), breaks the elegant molecular device that normally gives the edge to GTP. Receptors appear to promote GDP release normally from α_s-R231H, but trigger binding of GTP at a rate 25-fold lower than to α_s-WT (ref. 42).

Comparing the 3D structure of the trimer to that of Gα·GTP (Fig. 5) reveals the probable mechanism of the R231H activation defect. The arginine at position 231 (red in Fig. 5) is conserved in the α2 helix of all Gα proteins. Upon binding of GTP, this helix moves towards the guanine nucleotide and twists about its axis to form a coordinated complex with residues in the α3 helix and the preceding loop[17,18,22]. In this complex (Fig. 5b), the conserved arginine side chain forms a salt bridge with a conserved glutamate in the α3 helix, positioning α2 precisely with respect to α3; in addition, its guanidinium group stabilizes the main-chain oxygen of the same glycine, whose amide group interacts with the γ-phosphate of GTP. Mutations at either position of this conserved arginine–glutamate pair also inhibit activation of other trimeric G proteins[24,43]. The α_s-R231H phenotype suggests that the salt bridge serves as an intramolecular hasp to fasten together the α2 and α3 helices[42], allowing Gα to hold GTP tightly and maintain the active conformation more effectively (Fig. 5b).

The hormone-response defect in patients who inherit the α_s-R231H mutation results from failure of $G\alpha_s$ to complete the second step of the GDP/GTP exchange reaction. The broken hasp weakens the ability of Gα to stabilize the GTP-bound conformation required to disengage from Gβγ and the receptor. Consequently, GTP loses the tug-of-war against Gβγ for control of the α2 helix.

Perspective

We have outlined a speculative but comprehensive working model, in which the membrane-bound receptor uses two complementary mechanisms to act at a distance on the G protein's guanine-nucleotide-binding pocket. The model highlights unique features of the molecular mechanism crafted by evolution to regulate GDP/GTP exchange on trimeric G proteins. These proteins differ from their cousins, the monomeric GTPases, in many ways. Unlike Gα, the latter proteins show little or no preference for binding GTP over GDP, in part because they lack a hasp linking the α2 and α3 helices (as noted previously[27]).

In creating Gβγ as an adjuvant catalyst of GDP/GTP exchange, evolution generated four other important consequences for signal transduction: (1) in the absence of hormone, Gβγ reduces signal noise by stabilizing GDP binding[39,44,45], even though Gβγ is also required for transducing the hormonal stimulus; (2) GTP-dependent release of free Gβγ provides a second potential regulator of downstream effectors, in addition to Gα·GTP[39,45]; (3) receptor-dependent activation of Gα is irreversible, because the low affinity of Gα·GTP for Gβγ prevents it from interacting with the receptor; (4) the tighter association of GTP than GDP with Gα means that the transmitted signal cannot be terminated by dissociation of bound nucleotide, but only by its hydrolysis.

This model will certainly not prove correct in all its details. Eventually, G-protein-coupled receptors will be crystallized in their active and inactive forms and in association with $\alpha_e\beta\gamma$. In the interim, testing the model will require painstaking biochemical experiments with pure receptors and G-protein subunits. □

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Correspondence should be addressed to H.R.B. (e-mail: h_bourne@quickmail.ucsf.edu).